Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 846-854

Received: 18-06-2020
Accepted: 26-08-2020

KT Vijayakumar
All India Co-ordinated Research Project on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka, India

T Neethu
All India Co-ordinated Research Project on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka, India

NS Bhat
All India Co-ordinated Research Project on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka, India

T Nayimabanu
All India Co-ordinated Research Project on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka, India

H Varsharani
All India Co-ordinated Research Project on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka, India

Corresponding Author:
KT Vijayakumar
All India Co-ordinated Research Project on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka, India

Physico-chemical property of different floral honeys of Bangalore region, Karnataka

KT Vijayakumar, T Neethu, NS Bhat, T Nayimabanu and H Varsharani

Abstract
Honey is a product obtained from honey bee hives which has medicinal and antibacterial properties. The physico chemical properties of honey vary with bee species, floral source, climate, geography etc. The honey samples weighing about 50-100g were collected directly from beekeepers from different parts of Bangalore, Karnataka. Twenty three honey samples were tested for pollen diversity and physico-chemical properties. Pollen grains in the honey samples belonged to various plant families *viz*., Euphorbiaceae, Asteraceae, Myrtaceae, Meliaceae, Acanthaceae, Fabaceae (Leguminaceae), Malvaceae and Liliaceae. Number of pollen grains ranged between 15,000 and 7, 48,000 per 10 g of honey. Out of 23 honey samples, 19 were unifloral type and 4 were multifloral. The tested honey samples varied in their physic-chemical properties *viz.*, total solids (79.65±2.42%), RI (1.473 to 1.495), ash content (0.32±0.07%), seven colour variation (water white to dark amber), specific gravity (1.40±0.03), moisture content (20.35±2.42%), EC (0.86 ±0.42 dS/m), total reducing sugars (68.16% ±5.86%) and sucrose (2.94% ±1.26%). Eucalyptus honeys recorded highest TRS content irrespective of honey bee species.

Keywords: Physico-chemical, floral honeys, Acanthaceae

Introduction
Honey bees are well known and economically beneficial insects. Honey is the most valuable and natural product obtained from honey bees [1]. Honey is used in Ayurveda medicines as well. The major contributors in honey production (in 1000 metric tons) in the year 2018 are China (446.9), Turkey (114.11), Argentina (79.47), Iran (77.57), Ukraine (71.28), USA (69.1), India (67.44), Russia (65.01) and Mexico (64.25) (source: - statista.com). In India, the leading honey producing states are West Bengal, Uttar Pradesh, Punjab, Bihar, Kerala and Karnataka (source: - top10statesindia.com).

The flora of Karnataka is rich and diversified, which includes agricultural, plantation, commercial, horticultural crops and forest flora [2]. Karnataka is having a wide bio-diversity due to the presence of Western Ghats where we find plethora of unique bee plant species. Thus Karnataka is having a favorable environment for four species of honey bees and singles bees, in addition to other non-*Apis* bees. Bangalore which is known as garden city of India has many floras to nourish the pollinators of both wild and domestic bees.

Honey is one of the nature's gift to mankind. Honey is defined as the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature [3]. Generally, the physico-chemical properties of honey vary with type of honey bee species, type and nature of bee flora, seasons and geographical areas [2].

Melissopalynology (the study of pollen in honey) is an important tool in determining the geographical origin and floral sources upon which the bees foraged to produce honey. Each flower species has a unique pollen grain which, using proper techniques, may be studied to determine the geographical origin and major floral sources of the honey [4]. Since 2000, this technique is slowly getting popularized in India where few scientists attempted to identify the pollen diversity in honey samples.

Presence of pollen in honey is essential to establish its geographical origin. Importing countries such as USA through its organization like FDA evaluates the imported honey using pollen types. The diversity of pollen grains in honey varied with locations. In Brazil, *Eucalyptus* and *Citrus* sp. were predominant [5, 6]. With respect to India, honeys from Uttar Pradesh were predominant with pollen from *Antegonon* and *Moringa* [7], *Rumex*, *Nephelium*

sp. and members of Myrtaceae, Liliaceae, Rosaceae and Euphorbiaceae [8, 9]. Honey from Himachal Pradesh had preponderance of *Brassica, Adathoda, Clematis, Mussenda* and *Helianthus* sp. [10], *Citrus, Toona, Eucalyptus* and *Medicago* [11] pollen species in honey. Honey from Andhra Pradesh revealed that *Sapindus, Eucalyptus, Anacardium* and *Cleome* were major pollen types [12]. Nearly 142 pollen species were identified in the honey of *A. cerana* collected from Bangalore areas [13].

The honey bees in search of pollen sources fly up to a radius of 3 km. Hence it provides valuable information about honey source in that area. Honey is a biological product of the bee, made without human interference, which has great significance in maintaining human health [14].

Honey is completely a natural product and raw honey can be used directly from the comb taken from the bee hive. It is reported to contain about 200 substances comprising not only highly concentrated solution of sugars, but also the complex mixture of other saccharides, amino acids, peptides, enzymes, proteins, organic acids, polyphenols and carotenoid- like substances, vitamins, and minerals [15, 16, 17]. The keeping quality or shelf life of honey depends on HMF, sugars, acidity, diastase activity and development of microorganisms in stored honey. The development of these constituent affects the quality of honey in storage. The genuine honey develops HMF when heated or stored over long period of time in warm conditions. The loss of 10 to 20 per cent of diastase every month occurs in honey [18]. In honey, fructose in presence of acids produces Hydroxy Methyl Furfural (HMF). HMF is being considered as an indicator of the heat exposure of honey and also the adulteration with artificial sugar syrup that invariably contain more of HMF. Codex Alimentarius Commission (1969) has prescribed 40 ppm of HMF, whereas International Standards by Codex (1987) recommended HMF limits of 80 ppm. Indian Standards Institute (ISI 4941-1994) has now revised and specified the HMF limit to 80 ppm in honey [19]. With this background, different honeys were collected from various locations of Bangalore district, Karnataka and analyzed for its physico-chemical properties.

Materials and methods

The study pertaining to physico-chemical properties of honey was carried out at AICRP on Honey bees and Pollinators, Department of Apiculture, UAS, GKVK, Bangalore, Karnataka. Twenty-three samples were collected from parts of Bangalore (coded Ba) and Doddaballapura, Bangalore rural districts (coded Do), of which 13, 4, 4 and 2 samples were of *A. cerana, A. dorsata, Tetragonula* and *A. mellifera* respectively. The honey samples were coded with four or five letters where the first two letters stood for place (Ba or Do) of sampling, third letter for honey bee species (*A. cerana*-C; *A. dorsata*-D; *A. mellifera*-M, *A. florea*-F; *Tetragonula*-T), fourth letter for Pollen grain family and fifth and Sixth letter for genus of pollen. Places like Bangalore north, GKVK, Judicial layout, Hebbal, Palanahalli, Vijayanagara, Nagarabhavi, Rajanakunte and Doddaballapura were included for the collection. The observations made on density and diversity of pollen grains in honey collected from above places. The complete description on samples as mentioned in Table 1.

Collection of honey sample

The honey samples weighing about 50-100g were collected directly from beekeepers from different parts of Bangalore, Karnataka. Totally 23 honey samples were collected for this study. All samples were taken to the laboratory and stored in refrigerator at 4-5 ºC for further analysis. The honey samples were kept at ambient temperature overnight before the analysis were performed [20].

Melissopalynological studies

The pollen slides were prepared by the acetolysis method [21]. One gm of honey was dissolved in 9 ml of distilled water and centrifuged at 4000 rpm for 15 min. The pollen so obtained from this method was counted in all the 25 cells or squares of hemocytometer (consists 25 cells and 16 sub cells) under a microscope (Motic). The obtained counts were averaged and recorded.

The absolute pollen count (per 10 ml of suspension)=n X 10 X 10,000

Based on the percentage of each pollen present in the honey is classified as unifloral (more than 45% of single pollen) or multifloral. The grouping of honey was done taking into account pollen grains in 10 g honey as prescribed by [22]. Group I (<20,000pollen grains), Group II (20,000 - 100,000), Group III (100,000 - 500,000), Group IV (500,000 - 1,000,000) and Group V (>1,000,000) [23]. The images of pollen were captured by camera (Moticam 2300, 30 M Pixel, USB 2.0) attached to microscope. The pollen types were identified by comparison with reference slides of pollen collected directly from the plants in the study area. These pollen images were identified with the help of plant taxonomist (Dr.Sringeswara, taxonomist, UAS-B) [13, 24].

Refractive index (RI): Refractive index of honey was measured by using Abbe Refractometer (Rajdhani Scientific Instts. Co., New Delhi) at ambient temperature. Before the measurements, the Refractometer was calibrated using distilled water in accordance with the work manual of the instrument.

Moisture content: The moisture content of honey samples was measured by recording RI in Abbe Refractometer and correlating this with the moisture content. The moisture content of honey is expressed in per cent.

pH: Honey weighing 10g was dissolved in 50 ml of distilled water and the pH was recorded directly from the pH meter (Cyber pH-14 pH meter, Cyber lab).

Colour: The colour of honey was recorded by using honey colour analyzer (Hanna instruments) based on Pfund scale readings. The honey was taken in cuvette and placed in the holder provided in the instrument and readings of Pfund scale were recorded. Based on Pfund scales (mm) the honey colour was recorded according to the USDA classification.

Specific gravity: Specific gravity of honey was estimated using specific gravity bottle and taking weights of empty bottle (A), weight with water (B) and weight with honey sample (C). Then Specific gravity was calculated by using following formula

$$\text{Specific gravity at } 27\ ^{o}C = \frac{C\text{-}A}{B\text{-}A}$$

A= Mass of empty specific gravity bottle (g)
B= Mass of specific gravity bottle with water (g)
C= Mass of specific gravity bottle with honey sample (g)

Electric Conductivity (EC): Honey sample of 10 g was dissolved thoroughly in 25 ml of distilled water in a beaker and EC cell of Electric conductivity meter (Systronics) was dipped in the solution to record the electric conductivity. The reading was expressed in deci Siemen per meter (dS/m).

Total Soluble Solids (TSS): Total soluble solids of the honey samples were directly measured using Abbe Refractometer and the results were expressed in º Brix. All the measurements were done at ambient temperature and the readings were corrected for a standard temperature of 20 ºC by adding the correction factor of 0.00023 ºC [25].

Total Solids (TS): The Total Solid content (TS) in the honey samples were calculated using the formula.

TS (%) = 100-Moisture content

Total Reducing Sugars: Honey sample weighing one gm was dissolved in water and volume is made up to 250ml. Honey solution was taken in a burette and titrated against five ml of each copper sulphate solution and potassium sodium tartrate solution (Commonly called Fehling Solution A&B, respectively). Later it was heated over an asbestos gauge. Few drops of methylene blue indicator were added and continued the titration till the colour changed from blue to red. Titral reading was noted down [25].

$$\text{Total reducing sugar per cent by mass} = \frac{\text{250 x 100 x Strength of Copper sulphate solution}}{\text{Honey solution req. for titration (ml) x wt. of honey (g)}}$$

Sucrose: Honey sample weighing one gm was dissolved in water and volume is made up to 250ml. From this 100 ml of the honey solution was taken in a conical flask and one ml of concentrated hydrochloric acid was added to this and the solution was heated to near boiling. Sample was kept aside overnight. This inverted honey solution was neutralized with sodium carbonate (Himedia-4) and total reducing sugars was determined (as mentioned above in 3.6.1). Sucrose was calculated using below said formula [25].

Sucrose per cent by mass = [TRS after inversion in per cent by mass- TRS before inversion per cent by mass] X 0.95

Glucose: Honey sample weighing one gm was dissolved in water and volume is made up to 250 ml. From this, 50 ml of honey solution was pipetted out in stoppered flask. To this 40 ml of iodine solution and 25 ml of sodium hydroxide solution was added. Stoppered the flask and kept in dark for 20 minutes. Acidified with five ml of concentrated sulphuric acid and titrated quickly the excess of iodine against standard sodium thiosulphate solution. A blank using 50 ml of water also was run in place of honey solution [25].

$$\text{Glucose per cent by mass (W)} = \frac{\text{(B-S) x 0.004502 x 100}}{\text{A}}$$

B = Volume of Sodium thiosulphate solution required for the blank
S = Volume of Sodium thiosulphate solution required for the Sample
A = Mass of honey taken for test

Fructose: The fructose is directly estimated by using below mentioned formula [25].

$$\text{Fructose per cent by mass} = \frac{\text{Total Reducing Sugar percent - Glucose per cent by mass}}{0.925}$$

Hydroxy Methyl Furfural (HMF): Honey sample weighing 10 g is taken and dissolved without heating in 20 ml oxygen free distilled water. This is transferred to a 50 ml graduated flask and made up the volume. The sample is tested after preparation without delay. Two milliliter of honey solution is pipetted out into each of two test tubes and five ml of P-toluidine solution is added to each of this. Into one test tube one ml water and into the other one ml barbituric acid solution is pipetted and both mixtures are shaken thoroughly. The one added with water serves as water blank. The addition of the reagents should be done without pause and should be finished in about one to two minutes. The extinction of the sample is read against the blank at 550 nm using one cm cell, immediately after the maximum value is reached.
The method may be calibrated by using a standard solution of HMF standardized by dissolving commercial or laboratory prepared HMF and assaying Spectro-photometrically, where E=16.830 at 284 nm using 0.3000 µg standards.
An equation by which result may be worked out.

$$\text{mg/100 g HMF} = \frac{\text{Absorbance at 550 nm *19.2}}{\text{Thickness of cell layer (1cm)}}$$

Results were expressed as mg HMF/Kg of Honey
Ash content: The Ash content in the honey sample was estimated by burning 5- 10 g of honey sample with few drops of pure olive oil in a silica or platinum dish. Then Ignited in a Muffle furnace at 600±20ºC till white ash obtained and finally weighed [25].

$$\text{Ash percent by mass} = \frac{\text{100 (M2-M)}}{\text{(M1-M)}}$$

M=Mass in g of the empty dish
M1=Mass in g of the dish with the material taken for the test
M2= Mass in g of the dish with ash
Acidity: Honey sample weighing 10 g was taken in a suitable titration flask and dissolved in 75 ml of carbon dioxide-free water. Titrated against standard 0.05 N sodium hydroxide solution by using 4 to 6 drops by carefully neutralized phenolphthalein solution (pink colour of indicator should persist for at least 10 seconds). Determined blank on water and indicator and corrected the volume of standard sodium hydroxide solution used.

$$\text{Acidity (as formic acid) Percent by mass} = \frac{\text{0.23 x Volume of NaOH}}{\text{Mass of honey}}$$

Volume of NaOH= (Volume of NaOH required for sample titration) - (Volume of NaOH required for blank titration)

Results and Discussion
Melissopalynological studies
The diversity of pollen species in honey depended upon the nectar sources and their intensity available around the colony

which may change with the time and geographical location. The current study has documented variety of pollen grains in honey samples with specific to in and around Bangalore surrounding areas.

The pollen grains in the tested honey samples belonged to various plant families *viz.*, Euphorbiaceae, Asteraceae, Myrtaceae, Meliaceae, Acanthaceae, Fabaceae (Leguminaceae), Malvaceae and Liliaceae. The pollen in the samples belonged to floral species as *Suregada angustifolia*, *Coriandrum sativum*, *Caesalpinia coriaria*, *Asystasia gangetica*, *Synedrilla nodiflora*, *Syzygium cumuni*, *Eucalyptus* sp., *Azadirachta indica*, *Evolvulus alsinoides*, *Parthenium hysterophorus*, *Schinus terebinthifolius*, *Pongamia pinnata*, *Jatropha* sp., *Sweatna mahagoni*, *Coelogyna nervosa*, *Tabubia* sp., *Tamarindus* sp., *Glyricidia* sp. and to 9 unidentified plant species. Number of pollen grains ranged between 15,000 and 7, 48,000 per 10 g of honey. Based on this, out of 23 samples 8 belongs to group III, 8 to II, 6 to IV and 1 to I group respectively.Out of 23 honey samples, 19 were unifloral type and 4 were multifloral. Sample, BaTM/E had highest pollen count of 7, 48,000 and BaCM/E-1 with 15,000 had lowest count (Plate 1 and Table 1).

The study indicated geographical specialty of honey on the basis of major pollen types. The presence of pollen grains in the tested honey samples belonged to eight different plant families. Among the unifloral honeys with respect to different species of honey bees, *Eucalyptus* sp (8) was found predominant followed by *Pongamia pinnata* (3), *Azadirachta indica* (2), *Synedrilla nodiflora* (2), *Suregada angustifolia* (1) similarly in case of multifloral honeys, the major share was *Eucalyptus* sp. Similarly, pollens from *Cocos*, *Eucalyptus*, *Schefflara* and *Mimosa* species were found in honey from Karnataka [26]. In the honey of *A. cerana*, 142 pollen species collected from in and around Bangalore indicating multifloral nature of honey [13].

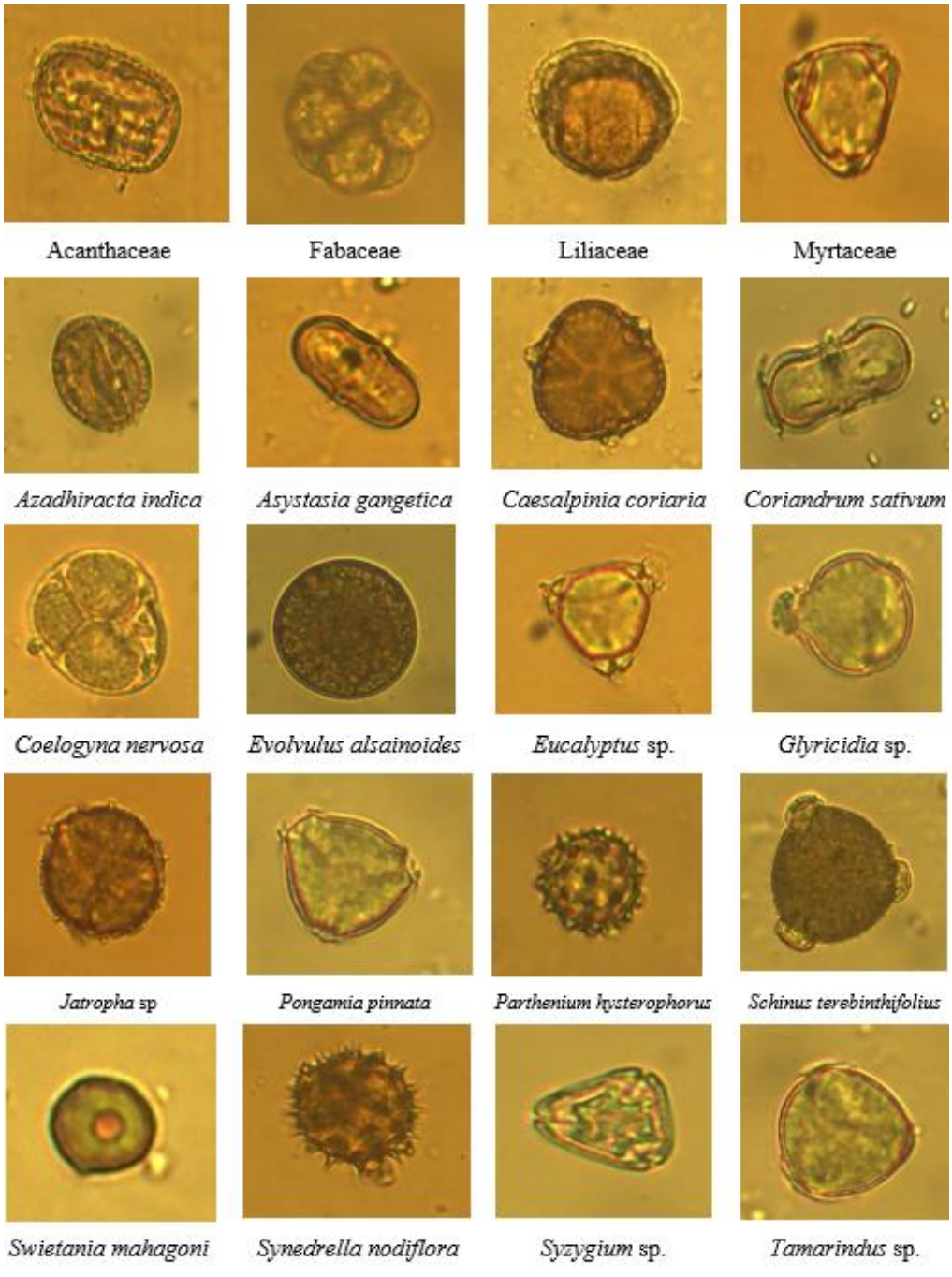

Plate 1: Diversity of pollen grains in honey collected from various locations of Karnataka

Table 1: Density and diversity of pollen grains in honey collected from Bangalore district

Sl. No.	Sample code	Description	Total Pollen count (per 10 g)	Pollen types	Major Pollen	Other pollens	Group
1	BaCE/Sa	Bangalore, *A. cerana*, Euphorbiaceae, *Suregada angustifolia*	3,55,000	5	*Suregada angustifolia*	Liliaceae, *Coriandrum sativum, Caesalpinia coriaria*, Unidentified-1	III
2	BaCMF-1	Bangalore, *A. cerana*, Multiflora	2,62,500	4		*Asystasia gangetica*, Liliaceae, Unidentified-2	III
3	BaCA/Sn-1	Bangalore, *A. cerana*, Asteraceae (Compositae), *Synedrella nodiflora*	97,000	2	*Synedrella nodiflora*	*Evolvulus alsinoides*	II
4	BaCMF-2	Bangalore, *A. cerana*, Multiflora	1,02,000	4		Acanthaceae, Liliaceae, *Parthenium hysterophorus, Schinus terebinthifolius, Synedrella nodiflora*	II
5	BaCF/Pp-1	Bangalore, *A. cerana*, Fabaceae (Leguminosae) *Pongamia pinnata*	63,100	2	*Pongamia pinnata*	Unidentified-3	II
6	BaCM/Ai	Bangalore, *A. cerana*, Meliaceae, *Azadirichta indica*	5,81,000	3	*Azadirichta indica*	Myrtaceae, Unidentified-4	IV
7	BaCF	Bangalore, *A. cerana*, Fabaceae	63,000	3	Fabaceae (Leguminaseae)	*Jatropha* sp, *Eucalyptus* sp.	II
8	BaDM/E-1	Bangalore, *A. dorsata*, Myrtaceae, *Eucalyptus* sp.	5,56,000	3	*Eucalyptus* sp.	Myrtaceae (May be *Syzygium* sp.), Unidentified-5	IV
9	BaTM/E	Bangalore, *Tetragonula*, *Eucalyptus* sp.	7,48,000	3	*Eucalyptus* sp.	*Evolvulus alsioides*, Liliaceae	IV
10	BaTM/Ai	Bangalore, *Tetragonula*, Myrtaceae, *Azadhiracta indica*	2,45,000	3	*Azadhiracta indica*	*Evolvulus alsinoides*, Fabaceae (Leguminasea	III
11	BaTL-1	Bangalore, *Tetragonula*, Liliaceae	1,48,000	3	Liliaceae	*Caesalpinia coriaria, Sweatna mahagoni*	III
12	BaTL-2	Bangalore, *Tetragonula*, Liliaceae	6,58,000	2	Liliaceae	*Caesalpinia coriaria*	IV
13	BaCF/Pp-2	Bangalore, *A. cerana*, Fabaceae (Leguminosae) *Pongamia pinnata*	32,000	3	*Pongamia pinnata*	*Eucalyptus* sp., *Jatropha* sp.	II
14	BaMMF-1	Bangalore, *A. mellifera*, Multiflora	1,92,000	3		*Caesalpinia* sp., *Coelogyna nervosa, Eucalyptus* sp.	III
15	BaCM/E-1	Bangalore, *A. cerana*, Myrtaceae, *Eucalyptus* sp.	15,000	1	*Eucalyptus* sp.		I
16	BaDM/E-2	Bangalore, *A. dorsata*, Myrtaceae, *Eucalyptus* sp.	5,16,000	3	*Eucalyptus* sp.	*Eucalyptus* sp., *Pongamia* sp., Unidentified-6,7	IV
17	BaDM/E-3	Bangalore, *A. dorsata*, Myrtaceae, *Eucalyptus* sp.	1,80,000	3	*Eucalyptus* sp.	Myrtaceae, *Tabubia* sp.	III
18	BaCM/E-2	Bangalore, *A. cerana*, Myrtaceae, *Eucalyptus* sp.	30,000	1	*Eucalyptus* sp.		II
19	BaCM/E-3	Bangalore, *A. cerana*, Myrtaceae, *Eucalyptus* sp.	1,63,000	4	*Eucalyptus* sp.	Liliaceae, Unidentified-8	III
20	BaMM/E	Bangalore, *A. mellifera*, Myrtaceae, *Eucalyptus* sp.	32,000	1	*Eucalyptus* sp.		II
21	BaCA/Sn-2	Bangalore, *A. cerana*, Asteraceae (Compositae), *Synedrella nodiflora*	1,43,000	2	*Synedrilla nodiflora*	*Eucalyptus* sp.	III
22	DoCMF-5	Doddaballapura, *A. cerana*, Multiflora	84,000	5		Fabaceae (Leguminaseae), *Ceasalpinia* sp., Malvaceae, Myrtaceae, Unidentified-9	II
23	BaDF/Pp	Bangalore, *A. dorsata*, Fabaceae (Leguminosae) *Pongamia pinnata*	5,21,500	3	*Pongamia pinnata*	*Tamarindus* sp., *Glyricidia* sp.	IV

Table 2: Physical characterizations of honey of different species of honey bees collected from Bangalore district

Sl. No.	Samples	RI @ 20 ºC	Moisture (%)	TSS (º Brix)	TS m(%)	P fund (mm)	Colour	Specific gravity	pH	EC (dS/m)
	***Apis cerana* (A)**									
1	BaCE/Sa	1.478	23.60	75.00	76.40	150	Dark Amber	1.39	4.21	0.96
2	BaCMF-1	1.483	21.60	77.00	78.40	150	Dark Amber	1.42	3.97	0.73
3	BaCA/Sn-1	1.483	21.60	77.00	78.40	150	Dark Amber	1.40	4.16	1.04
4	BaCMF-2	1.490	19.20	79.50	80.80	80	Light Amber	1.40	4.58	1.04
5	BaCF/Pp-1	1.479	23.20	75.50	76.80	90	Amber	1.40	4.19	0.84
6	BaCM/Ai	1.473	25.60	73.00	74.40	150	Dark Amber	1.38	4.29	0.96
7	BaCF	1.481	22.40	76.00	77.60	85	Light Amber	1.42	4.24	0.98
8	BaCF/Pp-2	1.480	22.80	75.50	77.20	93	Amber	1.35	4.46	0.73
9	BaCM/E-1	1.491	18.40	80.00	81.60	81	Light Amber	1.47	3.81	0.46
10	BaCM/E-2	1.492	18.00	80.50	82.00	114	Amber	1.42	3.56	1.31
11	BaCM/E-3	1.492	18.00	80.50	82.00	57	Light Amber	1.40	3.18	0.27
12	BaCA/Sn-2	1.489	19.20	79.00	80.80	76	Light Amber	1.37	3.65	0.40
13	DoCMF-5	1.494	17.20	81.00	82.80	57	Light Amber	1.41	4.47	0.27
	Mean	1.485	20.83	77.65	79.17	103		1.40	4.06	0.77
	S.D	0.007	2.65	2.58	2.65	36		0.03	0.41	0.33
	Max	1.494	25.60	81.00	82.80	150		1.47	4.58	1.31
	Min	1.473	17.20	73.00	74.40	57		1.35	3.18	0.27
	***A. mellifera* (B)**									

14	BaMMF-1	1.493	17.60	80.50	82.40	97	Amber	1.32	5.02	1.20
15	BaMM/E	1.495	16.80	80.50	83.20	81	Light Amber	1.36	4.13	0.69
	Mean	1.494	17.20	80.50	82.80	89		1.34	4.58	0.95
	S.D	0.001	0.57	0.00	0.57	11		0.02	0.63	0.36
	Max	1.495	17.60	80.50	83.20	97		1.36	5.02	1.20
	Min	1.493	16.80	80.50	82.40	81		1.32	4.13	0.69
	***A. dorsata* (C)**									
16	BaDM/E-1	1.487	20.00	78.50	80.00	98	Amber	1.39	3.91	1.14
17	BaDM/E-2	1.490	19.20	79.50	80.80	80	Light Amber	1.40	4.58	1.04
18	BaDM/E-3	1.491	18.40	80.00	81.60	52	Light Amber	1.45	3.79	1.27
19	BaDF/Pp	1.483	21.60	77.00	78.40	85	Light Amber	1.35	4.10	0.20
	Mean	1.488	19.80	78.75	80.20	79		1.40	4.10	0.91
	S.D	0.004	1.37	1.32	1.37	19		0.04	0.35	0.48
	Max	1.491	21.60	80.00	81.60	98		1.45	4.58	1.27
	Min	1.483	18.40	77.00	78.40	52		1.35	3.79	0.20
	***Tetragonula*(D)**									
20	BaTM/E	1.483	21.60	77.00	78.40	150	Dark Amber	1.41	3.73	1.23
21	BaTM/Ai	1.478	23.60	75.00	76.40	137	Dark Amber	1.38	4.27	1.96
22	BaTL-1	1.490	18.80	79.50	81.20	105	Dark Amber	1.40	4.36	0.60
23	BaTL-2	1.488	19.60	79.00	80.40	107	Dark Amber	1.41	4.02	0.51
	Mean	1.485	20.90	77.63	79.10	125		1.40	4.10	1.08
	S.D	0.005	2.15	2.06	2.15	22		0.02	0.28	0.67
	Max	1.490	23.60	79.50	81.20	150		1.41	4.36	1.96
	Min	1.478	18.80	75.00	76.40	105		1.38	3.73	0.51
	Grand total (A+B+C+D)									
	Mean	1.486	20.35	78.09	79.65	101		1.40	4.12	0.86
	S.D	0.006	2.42	2.28	2.42	32		0.03	0.40	0.42
	Max	1.495	25.60	81.00	83.20	150		1.47	5.02	1.96
	Min	1.473	16.80	73.00	74.40	52		1.32	3.18	0.20

Table 3: Chemical characterizations of honey of *Apis cerana*, *A. dorsata*, *A. melifera* and *Tetragonula* collected from Bangalore

Sl. No.	Samples	Ash %	TRS %	Sucrose %	Glucose %	Fructose %	HMF (mg/Kg)	Acidity%
***Apis cerana* (A)**								
1	BaCE/Sa	0.24	65.45	3.56	32.04	33.41	30.07	0.32
2	BaCMF-1	0.15	63.67	4.48	31.82	31.85	48.83	0.24
3	BaCA/Sn-1	0.38	68.32	5.34	33.36	34.95	36.84	0.17
4	BaCMF-2	0.25	69.96	3.53	34.05	35.92	27.29	0.19
5	BaCF/Pp-1	0.32	61.79	4.20	31.59	30.20	30.76	0.22
6	BaCM/Ai	0.31	69.57	2.58	33.16	36.41	41.51	0.08
7	BaCF	0.33	75.01	1.60	36.22	38.79	12.79	0.17
8	BaCF/Pp-2	0.21	72.07	1.83	34.93	37.13	22.35	0.31
9	BaCM/E-1	0.30	75.15	1.46	37.14	38.01	10.88	0.27
10	BaCM/E-2	0.31	69.98	1.27	33.11	36.87	32.57	0.15
11	BaCM/E-3	0.32	75.62	3.34	35.82	39.80	30.08	0.14
12	BaCA/Sn-2	0.28	71.85	2.73	34.45	37.40	7.83	0.20
13	DoCMF-5	0.27	63.24	2.31	31.34	31.91	23.13	0.20
	Mean	0.28	69.36	2.94	33.77	35.59	27.30	0.20
	SD	0.06	4.68	1.26	1.87	2.94	11.95	0.07
	Max	0.38	75.62	5.34	37.14	39.80	48.83	0.32
	Min	0.15	61.79	1.27	31.34	30.20	7.83	0.08
***Apis mellifera* (B)**								
14	BaMMF-1	0.33	68.56	5.70	33.59	34.97	5.12	0.16
15	BaMM/E	0.36	72.82	3.23	35.22	37.60	31.35	0.06
	Mean	0.35	70.69	4.47	34.41	36.29	18.23	0.11
	SD	0.02	3.01	1.74	1.15	1.86	18.55	0.07
	Max	0.36	72.82	5.70	35.22	37.60	31.35	0.16
	Min	0.33	68.56	3.23	33.59	34.97	5.12	0.06
***Tetragonula* (C)**								
16	BaTM/E	0.42	60.99	3.18	29.41	31.58	15.16	0.19
17	BaTM/Ai	0.44	56.56	3.45	30.54	26.02	16.20	0.17
18	BaTL-1	0.41	58.78	1.90	28.25	30.54	22.87	0.28
19	BaTL-2	0.42	58.41	2.25	26.82	31.60	26.54	0.16
	Mean	0.42	58.69	2.70	28.75	29.93	20.19	0.20
	SD	0.02	1.82	0.74	1.59	2.65	5.44	0.06
	Max	0.44	60.99	3.45	30.54	31.60	26.54	0.28
	Min	0.41	56.56	1.90	26.82	26.02	15.16	0.16
***Apis dorsata* (D)**								

20	BaDM/E-1	0.31	72.52	2.41	35.72	36.79	29.11	0.24
21	BaDF/Pp	0.40	70.38	2.25	33.32	37.06	9.69	0.28
22	BaDM/E-2	0.26	74.71	0.99	36.62	38.10	13.44	0.25
23	BaDM/E-3	0.25	72.27	4.15	34.36	37.90	10.88	0.17
	Mean	0.30	72.47	2.45	35.01	37.46	15.78	0.24
	SD	0.07	1.77	1.30	1.46	0.63	9.03	0.05
	Max	0.40	74.71	4.15	36.62	38.10	29.11	0.28
	Min	0.25	70.38	0.99	33.32	36.79	9.69	0.17
Grand Total (A+B+C+D)								
	Mean	0.32	68.16	2.94	33.17	34.99	23.27	0.20
	SD	0.07	5.86	1.26	2.67	3.47	11.51	0.07
	Max	0.44	75.62	5.70	37.14	39.80	48.83	0.32
	Min	0.15	56.56	0.99	26.82	26.02	5.12	0.06
	S.Em ±	0.02	0.54	0.55	0.47	0.69	1.86	0.03
	CD @ 1%	0.08	2.06	2.08	1.78	2.64	7.06	0.11

Physico-chemical Properties of honey

Twenty-three honey samples from *A. cerana* (13), *A. dorsata* (4), *Tetragonula irridipennis* (4) and *A. mellifera* (2) were subjected to analysis of various physical parameters *viz*., refractive index, moisture, total soluble solids, total solids, colour, Pfund values, specific gravity, pH and electric conductivity are presented in table: 2.

Refractive index of honey sample from Bangalore location showed variation from 1.473 to 1.495; the highest in *Eucalyptus-A.cerana* honey (BaMM/E) and lowest in *Azadhiracta indica-A. cerana* honey (BaCM/Ai). A study compared moisture content of *A. dorsata*, *A. cerana* and *A. mellifera* honeys and reported that it was more in the former followed by latter two [27]. Similar studies reported higher moisture content in *A. dorsata* honey compared to *A. cerana* and *A. florea* honeys [28]. But in present study, the moisture content of honey from *Teteragonula* was found higher followed by *A.cerana*, *A.dorsata* and *A.mellifera*. The moisture content of honey was determined based on the RI values where an average of 20.35±2.42% of moisture was estimated with the range of 16.80-25.6%. The moisture content and RI are inversely proportional. Moisture content depends on the botanical origin of the honey, harvesting season, degree of honey maturity, the degree of ripeness, processing techniques and storage conditions. Also, moisture content is affected by climate, season and moisture content of original plant nectar [29, 30].

Total soluble solids (TSS) of honey samples varied from 73º Brix to 81 º Brix in *Azadhiracta indica* (BaCM/Ai) and multifloral (DoCMF-5) samples respectively. Total solids in honey samples ranged from 74.40 to 83.20% with an average of 79.65 ±2.42%; being highest in *Eucalyptus* sp. (BaMM/E) and lowest in *Azadhiracta indica* (BaCM/Ai).

The colour of the honey samples of Bangalore varied light amber followed by amber and dark amber with Pfund values from 52 to 150 mm. Generally, honey of *Tetragonula irridipennis* was dark amber colour and that of *A. dorsata* light amber. Honey is classified by the U.S. Department of Agriculture into seven colour categories: water white (< 8mm), extra white (8 to 17 mm), white (17 to 34mm), extra light amber (34 to 50 mm), light amber (50 to 85 mm), amber (85 to 114 mm) and dark amber (>114mm). In this study, the majority (51.61%) of samples fell under light amber category followed by amber (22.58%) and dark amber (16.1%) colour. The substances responsible for colour of honey are largely unknown. However there was absence of tyrosine and tryptophan in light honeys, in contrast to their presence in dark honeys [18]. The colour in the tested honey was found dark amber in *Tetragonula irridipennis* with that of light amber in *A. dorsata*.

The specific gravity of honey was lowest of 1.32 in *A.mellifera* multiflora sample (BaMMF-1) and highest 1.47 in *Eucalyptus-A. cerana* honey (BaCM/E-1) with an average value 1.40±0.03. Electric conductivity value of honey samples ranged from 0.20 to 1.96 dS/m with an average of 0.86 ±0.42 dS/m. The Electrical conductivity (ds/m) of various honeys ranged from 0.196 to 1.96. The Electrical conductivity (ds/m) of various honeys ranged from 0.196 to 1.96. These findings are in agreement with that of another study which reported EC in the range of 0.33 to 0.94 mS/cm in Indian honeys [31]. The conductivity is strongly influenced by sugars, moisture and other insoluble materials [32] Electrical conductivity is closely related to the concentration of mineral and organic acids, shows great variability according to the floral origin [33, 34].

The pH of honey is always acidic range wherein the given samples it was estimated to be 3.18-5.02. All honeys showed less than 4.58 pH except *A. mellifera* honey i.e., BaMMF-1 sample which had pH of 5.02. A measure of the total concentration of hydrogen ions provides information on the strength of acidity and this is expressed in pH. The pH value is affected somewhat by the amounts of the various acids present, but mostly by the mineral content like calcium, sodium, potassium and other ash constituents. Honeys rich in ash generally show high pH values [35]. The ash content in the tested honeys showed an average of 0.32±0.07%.

The per cent ash content of honey samples collected from different species of honey bee showed range from 0.15 to 0.44% with average of 0.32±0.07%. Of which honey from *Tetragonula irridipennis* (BaTM/Ai, BaTM/E, BaTL-2 and BaTL-1) recorded significantly highest content of ash with ranged from 0.41 to 0.44% and these samples are on par *Pongamia-A. dorsata* honey (BaDF/Pp) (0.40%). Similarly,*A. cerana* honeys ranged from 0.15% to 0.38%, *A. dorsata* was0.25 to 0.40% and *A. mellifera* was 0.33 to 0.36%.

Total reducing sugar (TRS) in variety of honey showed significant variation among them. They were in ranged from 56.56 to 75.62% with a mean value of 68.16% ±5.86%. In case of *T. irridipennis* honeys, TRS content was minimum when compared with that of other samples i.e. 56.56%, 58.41%, 58.78% and 60.99% in BaTM/Ai, BaTL-2, BaTL-1 and BaTM/E respectively. It was found that highest TRS among *Apis cerana* honeys was found in *Eucalyptus-A. cerana* honey (BaCM/E-3) with 75.62%. With respect to *A. dorsata* and *A. mellifera* the maximum TRS was recorded in *Eucalyptus-A. dorsata* honey (BaDM/E-2) and *Eucalyptus-mellifera* honey (BaMM/E). Eucalyptus honeys recorded highest TRS content irrespective of honey bee species. The sucrose content in honey lot was ranged from 0.99 to 5.70%

with an average of 2.94% ±1.26%. It was found that the sucrose content was less than 5 per cent in majority samples except BaCA/Sn-1 (5.34%) and BaMMF-1 (5.70%). The results on glucose content revealed that the Eucalyptus honeys (BaCM/E-1, BaCM/E-3, BaDM/E-1 and BaDM/E-2) and Fabaceae honey (BaCF) were maximum significantly and they are on par with each other. Among the lot, stingless bee honey samples had least glucose content *viz*., BaTL-2 of 26.82% and 28.25% in BaTL-1. The lowest fructose found in *Azadirachta indica-T. irridipennis* honey BaTM/Ai with 26.02%; being highest in *Eucalyptus- A. cerana* honey (BaCM/E-3) with 39.80%. Stingless bee honey samples composed of significantly minimum fructose compared to that of *A. cerana, A. mellifera* and *A. dorsata.*

Honey is a supersaturated solution of various sugars. Sugars are the main constituents of honey, comprising about 95% of honey dry weight. Main sugars are the monosaccharide hexoses *viz*., fructose and glucose, which are products of the hydrolysis of the disaccharide, sucrose. Besides, about 25 different sugars have been detected in honey.

The hydroxy methyl furfural content of honey varied significantly among the samples however 9 samples were recorded less than 20 mg/kg of honey, 6 samples were in the range from 20 to 30 mg, 6 samples from 30-40 and 2 were more than 40 mg/kg. Significantly multiflora *A. cerana* honey (BaCMF-1) contained 48.83mg/kg which was found to be highest and followed by *Azadirachta indica- A. cerana* honey (BaCM/Ai) with 41.51 mg/kg and had least in multiflora *A. mellifera* honey (BaMMF-1) of 5.12 mg/Kg. In the current study higher HMF content in *A. dorsata* honey was observed over *A. cerana* honey which agrees with the other available studies [28, 36]. The continuous exposure of honey combs to atmosphere might have contributed to higher HMF in *A. dorsata*. In honey, mainly fructose, in presence of acids produces Hydroxy Methyl Furfural. HMF is being considered as an indicator of the heat exposure of honey and also as index of the adulteration with artificial sugar syrup that invariably contain more HMF. HMF is a break down product of fructose that increases with long storage, adulteration and excess of heating of honey. Long term effects of heat and storage cause increase in HMF in honey [37].

The acidity was varied from 0.06 to 0.32% in the given honey samples with average value of 0.20 ±0.07%. *Suregada angustifolia- A. cerana* honey (BaCE/Sa) and *Pongamia pinnata- A. cerana* honey (BaCF/Pp-2) had maximum acidity level whereas minimum was in *Eucalyptus- A. cerana* honey (BaMM/E).

Conclusion

The mellisopalynological studies concluded that honey samples were rich in pollen grains of *Eucalyptus* sp., followed by *Pongamia pinnata, Azadirachta indica, Synedrilla nodiflora, Suregada angustifolia*. BaTM/E had highest pollen count of 7, 48,000 and BaCM/E-1 with 15,000 had lowest count. The physico chemical properties gave the difference in each honey samples according to bee species and floral source. The moisture content of honey from *Teteragonula* was found higher followed by *A.cerana, A. dorsata* and *A.mellifera.* Eucalyptus honeys recorded highest TRS content irrespective of honey bee species. Stingless bee honey samples composed of significantly minimum fructose compared to that of *A.cerana, A. mellifera* and *A. dorsata.* Acidity level was maximum in *Pongamia pinnata- A. cerana* honey (BaCF/Pp-2) minimum in *Eucalyptus- A. cerana* honey (BaMM/E). The HMF contents in honey increases with the advancement of storage.

Acknowledgement

The authors acknowledge the project 'NAIP: Value chain on wild honey' at Department of Apiculture, College of Agriculture, GKVK, UAS, Bangalore for creation of honey analysis facilities and Dr. A. N. Sringeswara (Plant taxonomist, UAS-B) for identification of Pollen grains.

References

1. Vijayakumar KT, Neethu T, Shabarishkumar S, Nayimabanu taredahalli, Madhu KV, Bhat NS *et al*. Survey, Biology and Management of Greater Wax moth, *Galleria mellonella* L. in southern Karnataka, India, Journal of Entomology and Zoology Studies. 2019; 7(4):585-592.
2. Balasubramanyam, Reddy CC. Physical characteristics of multi-floral wild and apiary honey from plains, hills and Western Ghats of Karnataka. Indian bee Journal. 2003; 65(3 &4):113-119.
3. Mendes E, Brojo PE, Ferreira, Implvo, Ferreira MA. Quality evaluation of Portuguese honey. Carbohydrate Polymers. 1998; 37(3):219–223.
4. Petersen S, Bryant V. The study of Pollen and its role in the honey market, American Bee Journal. 2011; 151(6):591-594
5. Barth OM. Pollen in mono-floral honeys from Brazil. Journal of Apiculture Research, 1990; 29: 89-94.
6. Ramlho M, Guibu LS, Giannini TC, Kleinert A, Imperatriz VL. Characterization of some southern Brazilian honey and bee plants through pollen analysis. Journal of Apiculture Research. 1991; 30(2):81-86.
7. Nair PK, Singh KN. A study of two honey plants, *Antegonan leptopus* Hook and Martinga pterigosperma Gaetrn. Indian Journal of Horticulture. 1974; 31:375-379.
8. Sharma HK, Nair PKK. Pollen analysis of some honey from Uttar Pradesh. Indian Journal of Horticulture. 1965; 22:46-51.
9. Gaur RD, Nanwani P. A melittopalynological analysis of apiary honeys from Pauri Garhwal, Uttar Pradesh, India. Indian Bee Journal. 1989; 51:12-14.
10. Singh P, Verma LR, Mattu VK. Pollen spectrum of some honey of the North East Himalayas as a determinant of honey bee forage. Indian Bee Journal. 1994; 56:37-52.
11. Mattu N, Verma LR, Mattu VK, Verma AS. Palynological analysis of some autumn honeys from Himachal Pradesh, paper presented at National conference on Apiculture and First Karnataka honey festival, Bangalore, 1997.
12. Kalpana TP, Ramanujan CGK. Melissopalynology bee plants and beekeeping potential in some coastal districts of Andhra Pradesh, India. Indian Bee Journal. 1997; 59:1-8.
13. Sekhar P. Melissopalynological studies of *A. cerana indica* F. in Bangalore region, M.Sc. Thesis, University of Agricultural sciences, Bangalore, India, 2000.
14. Madejczyk M, Baralkiewicz D. Characterization of polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. Analytica Chimica Acta. 2008; 617:11–17.
15. Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agriculture Food

Chemistry. 2002; 50:5870-5877.
16. Sato T, Miyata G. The nutraceutical benefit, Part III. Honey Nutrition. 2000; 16:468-469.
17. White JW. Composition of honey. In E.Crane (Ed.), Honey: A Comprehensive Survey London: Heinemann, 1975, 157-206.
18. Phadke. Studies on Indian honeys-3, Proximate composition and physical chemical characterization of honeys from wild honey bee *Apis dorsata*, *Apis florea* and *Trigona*. Indian Bee Journal. 1968; 30:8.
19. Bogdanov S. Harmonized Methods of the International Honey Commission, responsible for the Methods, 1999, 1–54.
20. Saxena S, Gautam S, Sharma A. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chemistry. 2010; 118:391–397.
21. Erdtman G. Pollen morphology and plant taxonomy of Angiosperms. Chronica Botanica Co., Waltham, Massachusettes, 1952.
22. Louveaux J, Maurizio A, Vorwohl G. Methods of melissopalynology. Bee world. 1978; 59:139-157.
23. Xiao-Yan-Song, Yi-Feng Yao, Wu De Yang. Pollen analysis of natural honeys from the central region of Shanxi, North China, Plos one. 2012; 7(11):e49545
24. Louveaux J, Maurizio A, Vorwohl G. Methods of melissopalynology. Bee world. 1970; 51:125-138.
25. AOAC-Association of Official Analytical Chemists, Official methods of analysis. In K. Helrich (Ed.) (15th ed.). Arlington, VA, USA, Inc, 1990.
26. Singh TS, Suryanarayana MC. Melitopalynological investigations on honeys of Karnataka paper presented at National conference on Apiculture and first Karnataka honey festival, Bangalore, 1997.
27. Joshi SR, Pechhacker H, William A, Vonderohe W. Physico-chemical characteristics of *A. cerana*, *A. dorsata* and *A. mellifera* honey of Chitwan district, Central Nepal, Apidologie. 2000; 31:367-375.
28. Jyothi. Physico-chemical properties of honey from Bangalore region, Karnataka. Indian bee Journal. 2006; 64(1-4):18-22.
29. Estupinon S, Sanjuan E. Quality parameters of honey II chemical composition. Alimentaria. 1998; 297:117–122.
30. Finola MS, Lasagno MC, Marioli JM. Microbiological and chemical characterization of honeys from central Argentina. Food Chemistry. 2007; 100:1649–1653.
31. Chakir A, Romane A, Marcazzan GL, Ferrazzi P. Physico-chemical properties of some honeys produced from different plants in Morocco. Arabian Journal of Chemistry. 2011; 2:1-9.
32. Cozmuta AM, Cozmuta LM, Varga C, Marian M, Peter A. Effect of thermal processing on quality of polyfloral honey, Romanian Journal of Food Sciences. 2011; 1(1):45–52.
33. Ouchemoukh S, Louaileche H, Schweitzer P. Physico-chemical characteristics and pollen spectrum of some Algerian honeys. Journal of Food Control. 2007; 18:52–58.
34. Terrab A, Diez MJ, Heredia FJ. Characterization of Moroccan unifloral honeys by their physico-chemical characteristics. Food Chemistry. 2002; 79:373–379.
35. Crane E. Honey. Morrison and Gibb, London, 1975, 608.
36. Bhushanam M, Bhargava HR, Reddy MS. Antibiotic activity of various types of honey of Apis Species. World Journal of Agricultural Sciences. 2013; 9(4):309-315.
37. Sancho MT, Muniategui S, Huidobro JF, Lozano JS. Aging of honey, J. Agric. Food Chem. 1992; 40(1):134-138.